**Applicant Information**

- **Student Name:** Charles Thomas
- **Undergraduate:** ☑
- **Graduate:** ☐
- **Semester Hours Completed:** 54
- **UTC ID:** wjf783
- **Major:** Chemistry
- **UTC Email:** wjf783@mocs.utc.edu
- **Phone:** 423-364-3619
- **Home address:** 15 Brockhaven Rd., Chattanooga TN 37404
- **Faculty Sponsor:** Titus V. Albu
- **Department:** Chemistry

**Project Information**

- **Project Title:** Fluorescence Studies of Lysozyme Modifications Induced by Quinones
- **Project Start Date:** July 1, 2015
- **Project End Date:** Dec 31, 2015
- **Research Course (if applicable):** CHEM 4997
- **Compliance Requirements:**
  - ☐ Human Subjects (IRB review needed)
  - ☐ Use of animals (IACUC review needed)
- **Total Funding Requested (up to $1000):** $1,000

**Signatures**

- **Student Researcher:** Charles Thomas
- **Faculty Sponsor:** Dr. Titus V. Albu
- **Department Head:** Dr. Thomas Rybolt
**Fluorescence Studies of Lysozyme Modifications Induced by Quinones**

**Project Description/Research Objectives**

The objective of the proposed research is to investigate how lysozyme, a small-sized protein, is modified by the reaction with quinones. A better understanding of lysozyme modifications can possibly shine new light on understanding abnormal cell growth, also known as cancer. The proposed research will include conducting fluorescence studies of \( p \)-benzoquinone and other substituted quinones reacting with lysozyme as well as organizing and analyzing collected fluorescence data for the lysozyme modification induced by these quinones. The results of the study will be presented at the Southeastern Regional Meeting of the American Chemical Society, Inc. (SERMACS) in Fall 2015.

Quinones are six-membered carbon rings with two carbon-oxygen double bonds and two carbon-carbon double bonds within the ring itself. Examples of quinones that will be used in this study can be seen in Figure 1.

![Substituted quinone molecules](image)

**Figure 1:** Substituted quinone molecules that will be used in research: 1) \( p \)-benzoquinone 2) chloro-\( p \)-benzoquinone 3) methyl-\( p \)-benzoquinone
Quinones are toxic molecules that can exhibit their toxicity through redox cycling, adduct formation, and/or inducing enzyme polymerization.\textsuperscript{1,2} Redox cycling is initiated by quinone reduction by a reductase or a similar protein, which then is followed by oxidation by molecular oxygen. Adduct formation is the process of quinone attacking and attaching covalently to a protein. Quinone can also act as a link between proteins leading to protein oligomerization and polymerization. In addition, quinones are highly reactive and can also damage cells in the body. Lysozyme is a protein that belongs to a class of enzymes known as glycoside hydrolases. They are enzymes that damage bacterial cell walls by catalyzing hydrolysis of glycosidic bonds. Their main function is to work as anti-bacterial defense mechanisms in the body and studies have shown that lysozyme concentration increases in certain cancers.

**Project Significance**

Polycyclic aromatic hydrocarbons (PAHs) belong to a class of compounds that have been linked to the abnormal behavior of cells. These polycyclic aromatic hydrocarbons and their metabolites can specifically lead to protein aggregation. Studying PAH metabolites is interesting due to recent findings of the observed ribonuclease A modifications induced by \textit{p}-benzoquinone.\textsuperscript{1,2}

![Figure 2](image_url): Fluorescence data of RNase induced by \textit{p}-benzoquinone.\textsuperscript{2}
When proteins begin to aggregate, the result can disrupt encoding pathways leading to improper function of the cells. Protein aggregation occurs naturally as one gets older, and sometimes is the cause of Alzheimer’s, Parkinson’s, or Huntington’s disease.\textsuperscript{3-8} It can easily be understood how mature protein aggregation can be toxic; however, recent research has shown that \textit{immature} protein aggregations are much more toxic.\textsuperscript{9-10} In the proposed research we will look at the lysozyme protein and how it will be modified by certain PAH metabolites. The specific metabolites of interest are \textit{p}-benzoquinone and two of its derivatives, chloro-\textit{p}-benzoquinone and methyl-\textit{p}-benzoquinone.

\textbf{Research Design and Methods}

The proposed research involves taking solid \textit{p}-benzoquinone (or chloro-\textit{p}-benzoquinone or methyl-\textit{p}-benzoquinone) and making a solution of certain concentration within a phosphate buffer at pH=7.0. This solution will then be reacted with the lysozyme in a quartz cuvette. The modifications will be monitored at a predetermined wavelength inside the Horiba Jobin Yvon fluorimeter. The solution will be investigated at 37°C to simulate body temperature (since lysozyme is naturally found in the body). Excitation scans, along with anisotropy scans, will be conducted inside a batch file which will be run for 30 minute intervals for the first four hours, then in one hour intervals until 24 hours have passed. Once this data has been collected from the fluorimeter scanning, the solution will undergo dialysis in a Float-A-Lyzer from Spectrum Laboratories for 24 hours to remove all excess byproduct generated in the reaction (i.e. hydroquinone).\textsuperscript{2} After completion of dialysis, the solution will undergo one final scan at the 48-hour mark. Besides fluorescence monitoring, the reaction will also be investigated using UV-Vis spectroscopy.
Important factors to vary and monitor in conducting the research is the concentration of the substituted \( p \)-benzoquinones, the concentration of the lysozyme protein, and the fluorescence intensity. For example, if the solution is too concentrated, the fluorescence can be reduced in intensity. This phenomenon, called quenching, leads to inaccurate readings of the solution’s actual fluorescence intensity.

**Budget Justification**

I am requesting $1,000 of funding for my research. $300 of this amount will be used for presenting my research to 2015 SERMACS in Memphis, TN, including travel to and from Memphis, conference registration, lodging, and food expenses. The remaining $700 will be used for purchasing quartz cuvettes and chemicals (lysozyme and benzoquinones). Having more cuvettes, fluorimeter scans could be performed more efficiently. The chemicals are necessary to carry out the proposed research.

**Student Merit and Need**

Currently, I am learning how to operate the equipment I will be utilizing to conduct my research, including the Horiba Yvon fluorimeter and the UV-Vis. I have sufficient experience working in a laboratory setting, and I am quite proficient in doing so. By participating in the undergraduate research program my laboratory skills will be improved even more so. At the conclusion of my research I will have an even greater knowledge of lysozyme modification induced by quinones. Hopefully the results generated by this research will shine more light on abnormal cell growth and the effects PAH metabolites and substituted quinones have on proteins. This research will be carried out during the Summer 2015 semester and through the research course (CHEM 4997) during the Fall 2015 semester.
References


Budget Request

Please complete your budget in the form provided below. If you need additional space, the budget form can be continued on the next page.

### Personnel Expenses

Note: All personnel must be added to payroll in order to be paid for work completed.

<table>
<thead>
<tr>
<th>Name and Role of Student Researcher (add lines if multiple students are working on project)</th>
<th>Time Required (Student hours per week)</th>
<th>Time Period (e.g. summer, spring semester)</th>
<th>Funds Requested (whole dollars)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charles Thomas</td>
<td></td>
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</tbody>
</table>

### Fringe Benefits

Students Estimate @ 9% of wages requested above in Personnel Expenses

### Travel

Identify purpose, location, and duration of each trip, and show calculations for specific costs (e.g. airfare, mileage, lodging, meals) per current UT travel regulations.

<table>
<thead>
<tr>
<th>Regional ACS meeting in Memphis TN (travel, registration, lodging)</th>
<th>$300</th>
</tr>
</thead>
</table>

### Minor Equipment

Identify each minor instrument/piece of equipment needed to carry out proposed project.

- Quartz cuvettes: $500
- Chemicals (lysozyme and benzoquinones): $200

### Operating Expenses

Identify expenses such as printing and duplication, supplies, postage and telephone, maintenance and repairs, computer services, or other expenses and show calculations used to determine their costs.

### Total Project Costs Requested

$1000